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Dear Dr. Lederberg,

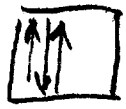
I have now collected some evidence of the matings in the crossing experiment and though a lot more work has to be done, I shall venture to give you a preliminary report. When you set the plates up at 5.0 the previous night, the matings turn up round about 11.0 the following morning, if incubation takes place at 22°C. This may not produce the maximum amount of matings. Higher temperature may be better (I shall have to examine this), but the time is rather convenient, I mean the time of the appearance of the matings. They have been observed by me in every experiment I have set up so far (about a dozen altogether). They seem to occur at a certain time; if the right time is missed everything seems to have blown over. Though I have found the matings in every experiment, they have not turned up in every preparation, far from it. Sometimes I have found almost 50% of the preps., fixed at a special time, positive, sometimes much less.. It appears to me that there is a definite relationship between the frequency of the matings and the amount of secondary growth later (colonies of prototrophs).

The method.

Impression preparations, fixed in osmic acid vapours and

and stained with Giemsa solution for several hours. Mounted eventually in the weak stain. Edged with paraffin wax, they keep for a day or so. No HCl treatment.

The appearance.

Preparations have to be searched, by going over them with high magn. in the way indicated by the arrows: 

Most of the fields have quite an ordinary appearance as shown in photo.1 and Fig.2. Yet suddenly, at any point of the search, a field may appear in which the organisms are completely changed. They are swollen, have no contours, the cytoplasm looks very thin, is very delicately stained and the nuclear structures are generally bigger and more lightly stained, more red, less dark-red or mauve. These are the organisms which are mating or rather going to mate. They generally ~~stretch~~ are extended over an area of several microscopical fields. At the edges of the area the organisms look only changed as described; in the middle they are usually in the process of mating. I think they coalesce and join up together. See the photos 2 - 5 and Fig.1., where you find the examples (Use magnifying glass resp. reading lens!). The mechanism is the same as the one that very often initiates the appearance of L forms. Indeed some of the configurations could be called L forms. I am also sending you a few photographs of early L form productions in a water vibrio and Fusiformis necrophorus for comparison.

However, in the crossing experiment L forms are only very

rarely found later on, and the reproduction of new bacilli must set in soon after the matings have occurred. Yet this part of the cycle I have not yet examined closely enough to be in a position of giving an opinion. Sometimes I have found "new microcolonies" in these later stages, but I am not sure yet about their significance.

I have sometimes only found one mating area in one preparation sometimes two and rarely three and often none.

The matings that occur in the crossing experiment can quite well be compared with what happens in Fusiformis necrophorus. I am convinced that what occurs in both cases is a complete change in the organisms followed by coalescence of those organisms that are in close touch; and then nuclear elements join up.

I have for some time been of the opinion that the L cycle may be something fairly normal in some organisms, and in other cases it seems to be aberrant. For example the development in Fusiformis necrophorus has always impressed me as being "normal" (of course: what is"normal"?), yet when the L cycle is provoked as for example when Li₂l or glycine or penicillin are used, then the development depends on the amount of these substances added to the media. In some cases the organisms show swellings induced by these means, and when the organisms "soften up", as they invariably do, the whole cytoplasm and nuclear structures may ooze out through a hole or spot of least resistance. Yet these masses may reorganize themselves into L forms and eventually

reproduce bacilli.

If I am right about the "matings" in the crossing experiment, then certainly there is a connection between what must be called the sexual cycle of bacteria and their L cycle. This is very ~~pleasing~~. It seems bacteria (in particular of the Coli group) have only one mechanism of fusion which is by means of "softening" and coalescing. Of course, the behaviour of the nuclear matter is still unknown, and a lot of other things should be investigated.

I only wish I could do this work in closer association with you. Anyhow, I hope I shall be able to come over in 1952!

Yours sincerely,

E. Klieneberger-Nobel.

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I know you will regard my letter as confidential & quite preliminary. —

E. N.